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Vitamin D, vitamin D receptor polymorphisms and breast cancer aggressiveness in African American and European American women

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14. ABSTRACT
In 579 newly diagnosed breast cancer cases and 574 healthy controls, we found serum 25-hydroxyvitamin D levels were lower in cases than in controls. Women with sufficient vitamin D levels were at lower risk of breast cancer (OR=0.37, 95% CI=0.27-0.51). The reduced risk of breast cancer in women with sufficient vitamin D levels was similar between premenopausal and postmenopausal women. When we further examined vitamin D levels in relation to breast cancer characteristics, premenopausal women with high vitamin D levels had lower risk of highly aggressive breast cancer vs less aggressive cancer, particularly triple negative breast cancer subtype (OR=0.27, 95% CI=0.10-0.71). There were no similar relationships found in postmenopausal women. These results suggested that low vitamin D levels may be associated with breast cancer risk. Among premenopausal women, low vitamin D levels may also be related with highly aggressive breast cancer characteristics.

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INTRODUCTION

The proposed research project is to 1) examine serum 25-hydroxy vitamin D levels and vitamin D receptor (VDR) genetic polymorphisms in association with breast cancer aggressive characteristics, and 2) examine the contribution of vitamin D and VDR polymorphisms to breast cancer racial disparity between African-American (AA) and European American (EA) women. The two objectives are addressed in a two-step approach using two different study populations. The first objective will be examined among breast cancer patients enrolled in the DataBank and BioRepository (DBBR) at Roswell Park Cancer Institute; the second objective will be nested in the Women's Circle of Health Study (WCHS), a large scale case-control study with both AA and EA women. By the end of the second year, we have completed the first objective by adding additional breast cancer cases and controls from DBBR based on what we had in the last progress report. We also completed part of the second objective by measuring vitamin D levels in healthy AA and EA women from WCHS, and genotyping of VDR polymorphisms have been done. We are now performing data analysis of the second objective. A revised manuscript form the first objective is in preparation for submission. The results from this study were used as a part of preliminary data for two large research grants, including the competitive renewal of the Pathways Study on evolutionary factors on breast cancer survivorship, and a PO1 grant on aggressive breast cancer characteristics in AA women.

BODY

As reported in last year's progress report, we submitted our findings on vitamin D levels and breast cancer aggressive characteristics to the Journal of Clinical Oncology for consideration of publication. Unfortunately, the manuscript was not accepted. One major critique was that we did not have a proper healthy control group for comparison with breast cancer patients. We decided to add an adequate number of controls and additional cases from DBBR to address the reviewers' concern. As shown in Table 1, we identified 574 healthy controls and 62 additional breast cancer cases. The cases were significantly older and had higher BMI than controls, which are consistent with previous findings in the literature. Table 2 summarizes tumor characteristics of the breast cancer cases included in the analysis. Most of the patients had early stage breast cancer, while more than 60% of the tumors were diagnosed at grade III. Majority of the women had estrogen receptor (ER) positive tumor, and 14.7% of women had triple negative subtype. The proportion of triple negative cancer in DBBR is similar to that reported in the literature.

Serum 25-hydroxyvitamin D (25-OHD) levels were measured by immunochemiluminometric assay on the DiaSorin LIASION automated instrument at Heartland Assays Inc. Based on 5% duplicates included blindly in the assays, the coefficients of variation was 8.8%. As shown in Table 3, serum levels of 25-OHD were much lower in cases than in controls (median, 22.8 vs 26.2 ng/ml, $p<0.01$). We classified women into vitamin D sufficiency status based on commonly used criteria. Among controls, only 38.5% has sufficient vitamin D levels, while 35.7% were vitamin D insufficient and 25.8% were deficient. The prevalence of vitamin D insufficiency and deficiency was even higher in breast cancer cases. These results confirmed the worsening epidemic vitamin D insufficiency in the US, particularly in the Western New York area. Considering the seasonal variations of vitamin D levels across a year, we fit a locally weighted polynomial regression model of measured levels and the week of blood collection time in a year. As shown in Figure 1, serum 25-OHD levels peaked during the summer time. We also examined vitamin D levels by age, BMI, and season of blood collection time among DBBR controls. As shown in Table 4, Women at older age appeared to have lower vitamin D levels but the difference was not statistically significant. There is strong negative correlation between BMI and vitamin D levels. Obese women had much lower levels than those with normal BMI.

Table 5 shows serum 25-OHD levels by tumor characteristics adjusted for age, BMI and season of blood collection. Patients with invasive breast cancer have much lower vitamin D levels than controls. Among breast cancer cases, those with higher tumor grade and ER negative status had slightly lower vitamin D levels than those with less aggressive disease, although the difference was not statistically significant. Patients with triple negative subtypes had a much lower vitamin D levels than those with luminal subtype (19.9±1.1 vs 23.0±0.5 ng/ml, p=0.02). After stratified by menopausal status, vitamin D levels were lower in women with invasive breast cancer than in controls, regardless of menopausal status (Table 6 and Figure 2). Among premenopausal women, those with highly aggressive tumors had significantly lower vitamin D levels than those with less aggressive tumors. Premenopausal women with triple negative breast cancer subtype had particularly low vitamin D levels (triple negative vs luminal (17.5±1.6 vs 24.3±0.7 ng/ml, p<0.001). Nevertheless, similar differences were not found among postmenopausal women.

We used logistic regression to estimate risk of breast cancer associated with vitamin D levels. As shown in Table 7, women with sufficient 25-OHD levels (≥30 ng/ml) were at much lower risk of breast cancer than those with deficient levels (<20 ng/ml) (OR=0.37, 95% CI=0.27-0.51). The relationship was similar in both premenopausal and postmenopausal women. We also examined the relationship with risk of breast cancer of different aggressive characteristics. Because the number gets smaller after categorizing on tumor characteristics, we dichotomized vitamin D levels into low and high based on the median in the controls. We found significant reduction in risk of breast cancer among postmenopausal women regardless of tumor characteristics. Among premenopausal women, however, the reduction of risk was most significant only in highly aggressive breast cancer (grade III, ER negative, and triple negative). We then conducted a case-only analysis (Tables 8 & 9). The results show among premenopausal women, high vitamin D levels were associated with reduced risk of highly aggressive vs less aggressive breast cancer. But such relationship was not observed in postmenopausal women.

AA women are more likely to have vitamin D deficiency than EA women. We measured 25-OHD levels in 242 AA and 187 EA women enrolled as controls in WCHS and adjusted for seasonality. As shown in Table 10 there were significant differences in 25-OHD levels by race, with AA women having an average mean level of 14.1 ng/ml and EA women averaging 22.2 ng/ml (p < 0.0001). BMI was inversely correlated with 25-OHD levels (r=-0.38, p<0.0001), and, because AA women in the WCHS had higher BMI than EA women (mean, 31.7 kg/m^2 vs 26.5 kg/m^2), we controlled for BMI in testing differences. After controlling for BMI and age, the racial differences in 25-OHD levels persisted (14.9 vs 21.4 ng/ml, p < 0.0001). AA women were also more likely to have severe vitamin D deficiency (<10 ng/ml) than EA women (34.3% vs 5.9%), a result similar to the national levels observed in NHANES data. If vitamin D is related to breast cancer subtypes, these striking differences in vitamin D levels could account, in part, for disparities in breast tumor biology between AA and EA women.

The genotyping of vitamin D receptor (VDR) and metabolism enzyme gene polymorphisms are undergoing and will be complete in summer 2010, followed by comprehensive data analysis. In addition, we also genotyped a panel of 51 SNPs that were identified from genome-wide association studies on skin pigmentation. These SNPs will be added to an algorithm to predict vitamin D levels of women in the WCHS, because serum samples are not available from them for vitamin D measurement. We will use these computated vitamin D levels to examine relationship of vitamin D and breast cancer characteristics among AA and EA women in the WCHS. This work will be completed in the third year of the study.

KEY RESEARCH AND TRAINING ACCOMPLISHMENTS

- We obtained additional pretreatment serum samples and data from 579 newly diagnosed breast cancer patients and 574 health controls from DBBR. Our analysis showed that serum 25-hydroxyvitamin D levels were lower in patients with breast cancer than controls in both premenopausal and postmenopausal women. When we further examined the relationship with breast cancer characteristics, we found premenopausal women with high vitamin D levels were less likely to have highly aggressive breast cancer, particularly the triple negative subtype, than those with low vitamin D levels.
- We measured serum 25-OHD levels in 242 AA and 187 EA healthy women enrolled in the WCHS. Our results showed that vitamin D levels were much lower in AA women than in EA women. The difference remained after controlling for BMI and age. The prevalence of severe vitamin D deficiency was almost 6-fold higher in AA than in EA women.

REPORTALE OUTCOMES

- A manuscript tilted “Serum 25-hydroxyvitamin D levels and breast cancer aggressive characteristics in premenopausal and postmenopausal women” is in preparation to be submitted to Cancer Research.
- Results generate from this study was used as a part of preliminary data in a competitive renewal of an RO1 grant the Pathways Study to investigate at vitamin D with breast cancer prognosis. This study has been recently funded by NCI. Our results were also used as a part of preliminary data in a PO1 grant to investigate evolutionary factors including vitamin D and pigmentation in relation to triple negative breast cancer in AA women. This grant is currently pending.

CONCLUSION

To conclude, we found premenopausal women with cancer of high aggressive characteristics including triple negative subtype, had much low serum 25-OHD levels than those with less aggressive cancers, indicating that vitamin D may prevent or delay breast cancer progression and reduce risk of breast cancer of high aggressive characteristics. A significant reduced risk of breast cancer was found in postmenopausal women with high vitamin D levels but there was no difference in vitamin D levels by tumor characteristics. The fact that the majority of the breast cancer patients are vitamin D deficient or insufficient at diagnosis confirms the epidemic vitamin D deficiency in the US, especially in breast cancer patients who may benefit from increasing vitamin D levels.

So what: Our results show vitamin D may prevent breast progression and reduce the risk of high aggressive breast cancer. If the results are further validated by a prevention trial, young women particularly those at high risk of developing breast cancer shall take vitamin D to prevent breast cancer occurrence and progression.

REFERENCES

None

APPENDICES

None

SUPPORTING DATA

Tables and Figures

Table 1. Descriptive characteristics of breast cancer cases and controls in DBBR

Characteristics	Cases (n=579)	Controls (n=574)
Age, mean ± SD (year)	55.8 ± 12.5	53.8 ± 13.8
Menopause, n (%)		
Premenopausal	245 (42.3)	245 (42.7)
Postmenopausal	334 (57.7)	329 (57.3)
BMI, mean ± SD (kg/m^2)	28.0 ± 6.2	27.8 ± 6.4
BMI category, n (%)		
<25.0 kg/m^2	177 (31.5)	184 (33.0)
25.0-29.9 kg/m^2	195 (34.7)	198 (35.5)
≥30.0 kg/m^2	190 (33.8)	175 (31.4)

Table 2. Tumor characteristics of breast cancer cases in DBBR

Characteristics	Cases (n=579)
Tumor stage, n (%)	
In situ	73 (13.0)
I/II	438 (77.8)
III/IV	52 (9.2)
Histological grade, n (%)	
Well differentiated	42 (8.9)
Moderate differentiated	124 (26.3)
Poorly differentiated	305 (64.8)
ER status, n (%)	
Positive	395 (76.4)
Negative	122 (23.6)
Molecular subtype, n (%)	
Luminal (ER+ and/or PR+)	402 (79.0)
Her2 overexpressing (ER-, PR- and Her2+)	32 (6.3)
Triple negative (ER-, PR-, and Her2-)	75 (14.7)

Table 3. Serum 25-hdyroxyvitamin D levels by cases and controls

Characteristics	Cases (n=579)	Controls (n=574)
Serum 25-OHD levels, median (range) (ng/ml)	22.8 (2.5-57.3)	26.2 (3.1-74.3)
Vitamin D sufficiency, n (%)		
Deficient (<20.0 ng/ml)	223 (38.5)	148 (25.8)
Insufficient (20.0-29.9 ng/ml)	232 (40.7)	205 (35.7)
Sufficient (≥30.0 ng/ml)	124 (21.4)	221 (38.5)

Table 4. Serum 25-OHD levels by demographic characteristics among DBBR controls

Characteristics	N (%)	Median (IQR), ng/ml	P-value
Age, year			0.56
<50	202	28.3 (19.8-36.3)	
50-59	169	27.0 (19.2-33.4)	
60-69	127	26.8 (19.4-32.3)	
≥70	76	26.7 (19.8-33.5)	
BMI, kg/m^2			<0.001
<25	184	30.7 (24.4-38.8)	
25.0-29.9	198	27.5 (20.7-33.2)	
≥30	175	21.6 (15.4-28.2)	
Season of blood collection			<0.001
Spring	99	25.7 (15.9-33.2)	
Summer	175	30.5 (22.9-36.9)	
Fall	135	25.2 (19.5-32.8)	
Winter	165	24.7 (16.5-31.8)	

Table 5. Serum 25-OHD levels by tumor characteristics

Tumor characteristics	N	Least square mean ± standard error (ng/ml)	P-value
Invasiveness			<0.001
Control	574	27.2 ± 0.4	
In situ	73	25.7 ± 1.2	
Invasive	506	22.7 ± 0.5	
Histological grade			0.16
Well/moderate differentiated	166	23.2 ± 0.7	
Poorly differentiated	305	22.0 ± 0.5	
ER status			0.06
Positive	380	22.9 ± 0.5	
Negative	116	21.1 ± 0.9	
Molecular subtype			0.02
Luminal (ER+ and/or PR+)	387	23.0 ± 0.5	
Her2 overexpressing (ER-, PR-, and Her2+)	32	21.6 ± 1.6	
Triple negative (ER-, PR-, and Her2-)	74	19.9 ± 1.1	

Table 6. Serum 25-OHD levels by tumor characteristics and menopausal status

Characteristics	Premenopausal			Postmenopausal		
	N	LS mean ± se	P-value	N	LS mean ± se	P-value
Invasiveness			0.001			<0.001
Control	245	27.1 ± 0.7		329	27.1 ± 0.6	
In situ	29	27.4 ± 2.0		44	24.7 ± 1.5	
Invasive	216	23.5 ± 0.7		290	22.2 ± 0.6	
Histological grade			0.005			0.81
Well/mod. differentiated	56	26.0 ± 1.3		110	21.9 ± 0.8	
Poorly differentiated	137	21.6 ± 0.8		168	22.1 ± 0.7	
ER status			0.01			0.76
Positive	155	24.2 ± 0.8		225	22.1 ± 0.6	
Negative	56	20.2 ± 1.3		60	21.7 ± 1.2	
Molecular subtype			<0.001			0.91
Luminal	160	24.3 ± 0.7		227	22.1 ± 0.6	
Her2 overexpressing	15	21.7 ± 2.5		17	21.2 ± 2.2	
Triple negative	34	17.5 ± 1.6		40	21.8 ± 1.4	

Table 7. Odds ratio and 95% confidence interval of breast cancer risk by vitamin D levels

Serum 25-OHD levels	All women		Premenopausal		Postmenopausal	
	# case/control	OR (95% CI)	# case/control	OR (95% CI)	# case/control	OR (95% CI)
Deficient	220/156	1.00	82/74	1.00	138/82	1.00
Insufficient	241/203	0.81 (0.61-1.08)	110/83	1.13 (0.72-1.77)	131/120	0.64 (0.44-0.94)
Sufficient	118/215	0.37 (0.27-0.51)	53/88	0.57 (0.34-0.93)	65/127	0.29 (0.19-0.45)
P-value for trend		<0.001	0.03			<0.001

Table 8. Odds ratio and 95% confidence interval of breast cancer risk by tumor characteristics

Serum 25-OHD levels	Histologic grade I/II		Histologic grade III		Histologic grade III vs I/II	
	# case/control	OR (95% CI)	# case/control	OR (95% CI)	# case/case	OR (95% CI)
Premenopausal						
Low	29/122	1.00	95/122	1.00	95/29	1.00
High	27/123	0.92 (0.49-1.74)	42/123	0.46 (0.29-0.74)	42/27	0.45 (0.22-0.91)
Postmenopausal						
Low	80/164	1.00	123/164	1.00	123/80	1.00
High	30/165	0.38 (0.23-0.61)	45/165	0.36 (0.24-0.54)	45/30	0.93 (0.54-1.63)
Serum 25-OHD levels	**ER positive**		**ER negative**		**ER- vs ER+**	
	# case/control	OR (95% CI)	# case/control	OR (95% CI)	# case/case	OR (95% CI)
Premenopausal						
Low	93/122	1.00	41/122	1.00	41/93	1.00
High	62/123	0.71 (0.45-1.11)	15/123	0.34 (0.17-0.67)	15/62	0.44 (0.21-0.93)
Postmenopausal						
Low	165/164	1.00	43/164	1.00	43/165	1.00
High	60/165	0.38 (0.20-0.69)	17/165	0.37 (0.25-0.53)	17/60	1.05 (0.54-2.04)
Serum 25-OHD levels	**Luminal**		**Her2 overexpressing**		**Triple negative**	
	# case/control	OR (95% CI)	# case/control	OR (95% CI)	# case/control	OR (95% CI)
Premenopausal						
Low	95/122	1.00	10/122	1.00	28/122	1.00
High	65/123	0.74 (0.47-1.14)	5/123	0.36 (0.11-1.15)	6/123	0.21 (0.08-0.53)
Postmenopausal						
Low	166/164	1.00	14/164	1.00	28/164	1.00
High	61/165	0.37 (0.25-0.54)	3/165	0.19 (0.05-0.70)	12/165	0.42 (0.20-0.86)

Table 9. Case-only analysis of breast cancer subtypes by vitamin D levels.

Serum 25-OHD levels	Her2 overexpressing vs luminal		Triple negative vs luminal	
	# case/case	OR (95% CI)	# case/case	OR (95% CI)
Premenopausal				
Low	10/95	1	28/95	1
High	5/65	0.44 (0.13-1.54)	6/65	0.27 (0.10-0.71)
Postmenopausal				
Low	14/166	1	28/166	1
High	3/61	0.54 (0.14-2.00)	12/61	1.14 (0.53-2.44)

Table 10. Measured serum 25-OHD levels in African American and European American controls in WCHS

Race	Unadjusted levels, ng/ml	Adjusted levels, ng/ml
AA	14.1 ± 0.5	14.9 ± 0.5
EA	22.2 ± 0.7	21.4 ± 0.6
P-value	$p < 0.001$	$P < 0.001$

Figure 1. Seasonal variations of serum 25-OHD levels

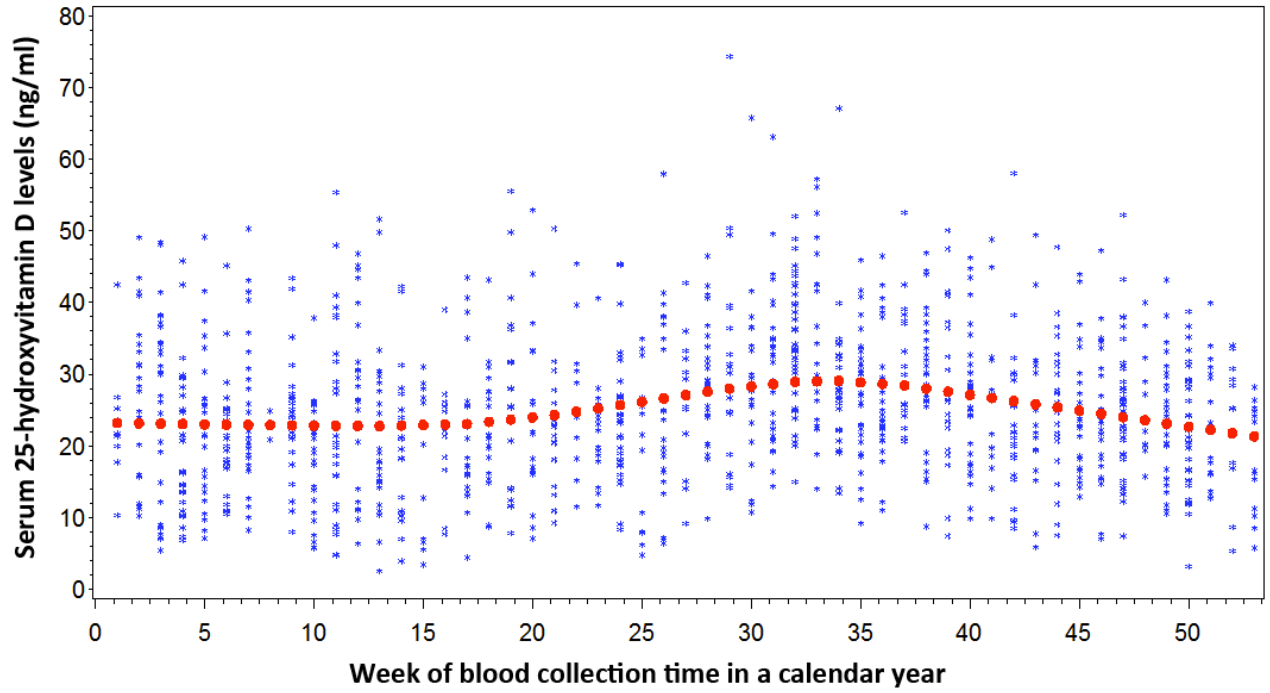

Figure 2. Serum 25-OHD levels by tumor characteristics and menopausal status